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# Kinetic Behavior of Electron Paramagnetic Resonance Signal I. II.[1] Comparison of Wild Type and Mutant (ac-206) *Chlamydomonas reinhardtii.*

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*Abstract.* The electron paramagnetic resonance (EPR) characteristics of wild type *Chlamydomonas reinhardtii* are compared with those of a mutant strain (ac-206) which lacks cytochrome 553. The steady-state signals I and II are similar but differ in their responses to light of long and short wavelengths, reflecting the fact that the electron transport chain linking photosystems I and II is interrupted. The kinetic behavior of signal I is simpler in the mutant, which lacks induction effects prominent in the wild type. The decay of the signal when light ceases is not dependent on the length or intensity of illumination in the mutant, whereas it is in the wild type. These data can be interpreted in terms of signal I being a reflection of cyclic flow in a pathway which does not involve cytochrome 553 in the mutant, whereas in the wild type there is also a contribution of electrons from photosystem II.

Preceding papers (7, 8) have described induction effects in the formation of electron paramagnetic resonance (EPR) signal I in intact algae of 3 species. This signal is attributed to the oxidized form of the photoreactive center for system I (P700); its kinetic behavior provides a sensitive tool for the observation of the state of P700. It was concluded that the dependence of the rate of rise of the signal on the length of dark time preceding the onset of illumination (induction effect) was largely a property of photosystem I. This conclusion was based on the observation that adding 3-(3,4-dichlorophenyl)-1, 1-dimethyl urea (DCMU) to whole cells had little effect on the induction curves, and that mutants of both *Scenesdesmus* and *Chlamydomonas* which lacked a functioning photosystem II still displayed prominent induction effects. Vredenberg and Amesz (6) had also found that the rate of bleaching of P700 in intact algal cells was strongly dependent on the length of the preceding dark period and was unaffected by DCMU. This correspondence was one of the bases for the contention that formation of EPR signal I and bleaching of P700 were the same process.

Levine and Gorman (2, 4) have reported a mutant of *Chlamydomonas reinhardtii* (ac-206) which lacks optically or functionally detectable cytochrome 553, which appears to be identical to cytochrome *f* found in higher plants, and they have kindly made this culture available to us. They have demonstrated that cytochrome 553 is an integral part of the intermediate electron transport chain, but that it is not essential to NADP photoreduction when the source of electrons is reduced 2,6-dichlorophenolindophenol. However, the absence of cytochrome 553 isolates the 2 photosystems from each other and we have demonstrated how this fact is reflected in the steady-state signal I and in its kinetic behavior.

## Materials and Methods

Wild type (Sager's 21 gr) and mutant ac-206 strains were grown in shake culture on mineral medium supplemented with 0.5 g per liter sodium acetate and 0.25 g per liter yeast extract. The temperature was 25° and light intensity 7000 lux. The cells were harvested after 5 or 6 days of growth and suspended in tris-HCl, 0.05 M, pH 7.2. Photosynthetic competence was tested with a Yellow Springs Instrument Company (YSI) Biological Oxygen Analyzer, Model 53. Chlorophyll was determined in a Cary 14 spectrophotometer in an 80 % acetone extract, using the method of Arnon (1). EPR techniques were the same as those described previously (6), although a new instrument (Varian 4500) with a 9-inch magnet was employed at X-band. A modulation amplitude of 4.45 gauss was routinely employed. The response time used was 1 sec for field scans, which were made at a rate of 20 gauss per min, and 0.1 to 0.01 sec for kinetic traces. During the latter, a larger modulation amplitude (10.5 gauss) was occasionally used to enhance signal-to-noise ratio. Light was provided by a system consisting of 2 separate lamps, described in detail elsewhere (5), whose output could be superimposed. Baird-Atomic interference filters (type B-1) with 10 nm width at half transmission height isolated desired spectral bands; 703 nm was

[1] Paper I of this series is E. C. Weaver, Photochem. Photobiol. 7: 93–100 (1968).

used to preferentially excite photosystem I, 633 nm for photosystem II. For saturation studies, a wider interference filter (type B-3) was used with 30 nm half width centered about 680 nm. Calibrated neutral density filters (Special Optics) attenuated the light intensity, which was measured in the EPR cavity with a Yellow Springs Instrument Company Radiometer, model 65, fitted with a special probe designed to fit in the cavity. Solenoid-activated shutters were either controlled by an automatic timing mechanism or manually switched. No computer averaging was employed.

## Results

Steady state signals I and II from mutant and wild-type cells were essentially identical; there was no variation in shape, $g$-value, or width. However, in the wild-type, the amplitude of the steady state signal produced by system I light could be decreased by superimposing system II light because electrons flowing from photosystem II reduced the number of oxidized photoreactive centers. In contrast, the steady state signal of the mutant excited by 703 nm light was increased by the addition of 633 nm light (Fig. 1).

The size of the total signal I when saturated by 680 nm light ($1.75 \times 10^5$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$) was slightly smaller for the mutant (87 % of wild type) on a chlorophyll basis. In addition to an absence or inactivity of cytochrome 553, mutant ac-206 had only 0.1 mg total chlorophyll per $10^8$ cells, whereas the wild type had twice as much.

Adding of DCMU (to 10 $\mu$M) to wild type cells greatly increased the signal when the system II light (633 nm) was used for illumination (3); however, adding DCMU to the mutant did not change the size of the signal produced by 633 light. DCMU produced little or no augmentation of signal size in either strain when the exciting wavelength was 700 nm or longer. The size of the signal in the mutant was usually a little smaller for a given intensity when the light was 633 nm rather than 703 nm. The difference, however, is much less marked than for wild type, in which the intensity of 633 nm light must be many times that of 703 nm in order to produce a comparable signal.

Rise kinetics presented a contrast between wild type and mutant cells (Fig. 2). The rise pattern of wild type cells is distinguished by a rapid initial jump, followed by a slow rise to the steady state, provided the preceding dark period was some tens of seconds long. The only effect of DCMU, which blocks the flow of electrons from photosystem II, is

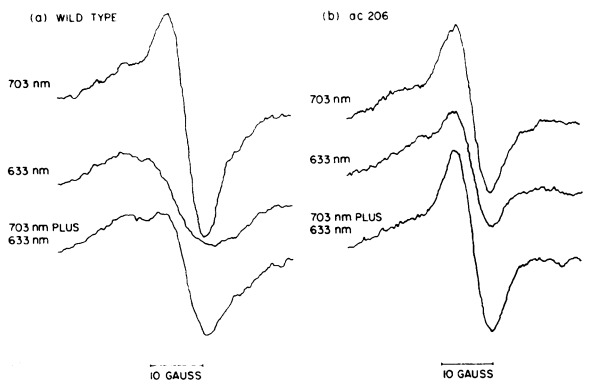


FIG. 1. Traces of the steady state light-induced EPR signals in suspensions of whole *Chlamydomonas* cells. The illumination is as indicated, with the intensity of the 703 nm beam incident on the sample cuvette $3.4 \times 10^3$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ and of the 633 nm beam $2.7 \times 10^3$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$. In the bottom traces, the 2 beams are combined. Chlorophyll concentrations: wild type, 0.82 mM, ac-206, 0.73 mM.

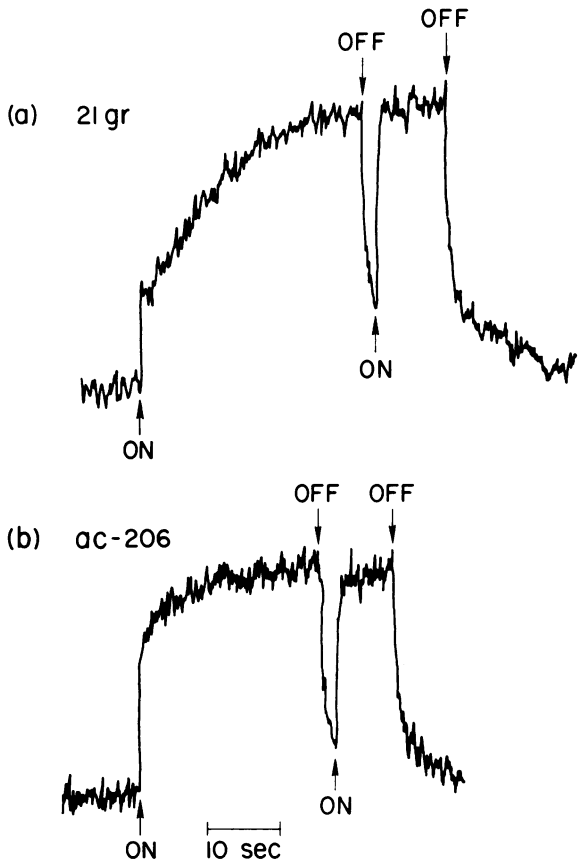


FIG. 2. Kinetic behavior of wild type and ac-206 strains. The magnetic field was fixed on the low-field peak of the first derivative. Light was 703 nm, intensity $7 \times 10^3$ ergs $\cdot$ cm$^{-2}$ $\cdot$ sec$^{-1}$ incident on the sample. Time constant, 0.1 sec. (a) Wild type, chl $a$ 0.73 mM. Light was turned on 40 sec after the previous illumination, left on for 34 sec, off for 2 sec, then on for 10 more sec. (b) ac-206, chl $a$ 0.62 mM. Light was turned on 55 sec after the preceding dark period, left on for 25 sec, off for 2 sec, then on for 8 more sec.

to eliminate the typical spike or short plateau with the onset of light. After a dark period of only 2 sec, the steady state is reached much more quickly than it was after the longer dark period. This dependence of rate of signal formation on the length of preceding darkness is the induction effect. The rate of signal formation in ac-206 (Fig. 2b) is almost independent of the length of the preceding dark period; that is, the induction effect is absent. Fig. 3 illustrates the contrast in the effect of the length of the dark period on the size of the signal formed during the first 10 sec of illumination for the 2 strains.

Decay kinetics are similar in wild type and mutant cells if the light which produces the EPR signal has been on several sec or more. Typically, most of the signal decays within approximately 0.5 sec, the last 20 % following a much slower first-order decay fith a $t_{1/2}$ of 50 or 60 sec, and decay characteristics are independent of light intensity or wavelength.

However, if the signal is produced by a 0.5 sec pulse of light, there is a marked difference in the mutant and wild type (Fig. 4). The signal from the mutant decays with the same characteristics as it shows after longer illumination. The height of the signal is proportional to the light intensity. On the other hand, the decay rate of the wild type signal is very dependent on the intensity of the flash. If the flash is bright, decay is rapid; if the light is dimmer, decay is slower. Consequently, the height of the flash-produced signal above the baseline is relatively independent of flash intensity.

## Discussion

Cytochrome 553 donates electrons coming from photosystem II to the photoreactive center of photosystem I, probably with the intervention of plastocyanin (2, 4). Its absence interrupts the electron transport chain between the 2 photosystems, but does not prevent the photoreduction of NADP with reduced 2,6-dichlorophenol-indophenol (4). The fact that the EPR signal does rise and decay indicates that the electrons cycle with little change in their behavior despite the lack the cytochrome 553. If the unpaired electrons simply recombined, one would expect very rapid second-order kinetics, which is not the case.

However, the lack of cytochrome 553 does abolish induction effects, in contrast to the action of DCMU, which does not, although both block electron flow from photosystem II. It has been suggested that induction effects in the oxidation of the photoreactive center are due to a pool of some substance which

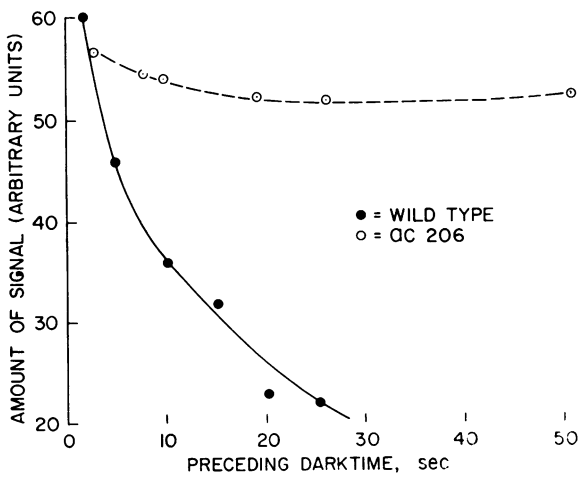


FIG. 3. Induction effect. The area under the rise curve for the first 10 sec of illumination is plotted as a function of the length of time in the dark preceding the illumination.

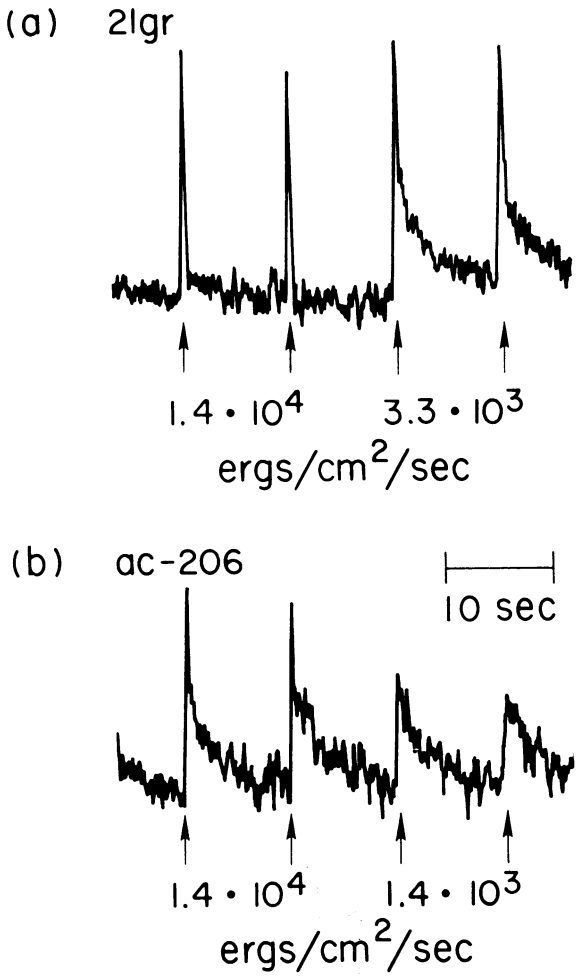


FIG. 4. Spikes resulting from giving 0.5 sec flash of 703 nm light every 10.5 sec. (a) Wild type. Decay time is rapid following a full intensity flash, and slow after an attenuated flash. (b) ac-206. Decay times of full intensity and attenuated light do not differ. Only the fast component of the former is lacking.

becomes reduced in the dark, and which lies on the electron transport pathway between the 2 photosystems (6, 7). The EPR behavior of mutant ac-206 lends support to this thesis, and localizes such a pool between the site of the DCMU block and cytochrome 553.

Rise kinetics in the wild type cells also fit the picture of such a reducing pool. After a relatively long time in the dark, the onset of light produces a rapid initial oxidation, which is followed by a transient decay, and then by a slower rise to the steady state when the light remains on. The transient can be visualized as being due to the arrival of electrons from the pool at the photoreactive centers. An explanation for the differing decay rates with pulsed light (703 nm) of high and low intensities can be suggested also. A low-intensity light produces the initial oxidation only, and does not suffice to start the flow of electrons from the intermediate pool; decay after a low intensity pulse is therefore slow. However, a bright pulse of light stimulates not only photosystem I but also photosystem II, and the electrons from the latter quickly reduce the oxidized P700, resulting in fast decay after a bright pulse. When the light has been on long enough to establish a steady state, these differences are no longer evident. The lack of cytochrome 553 prevents the wild-type "spike" and also the differences in decay rate with pulses of differing intensity.

The variables affecting signal kinetics in wild type cells are many and are currently under investigation; for the present, our purpose is to point out the simplification of kinetic behavior resulting from the lack of a component essential to overall photosynthesis. Two reasonable theories are supported; first, that the pool providing electrons responsible for the induction effect lies between the 2 photosystems, and not on the return pathway which is common to both mutant and wild type; and second, that the return cyclic pathway intersects with the intermediate electron pathway between cytochrome 553 and P700.

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